

Laboratory diagnosis of sepsis



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CDC

- Fever, increased heart rate, and chills

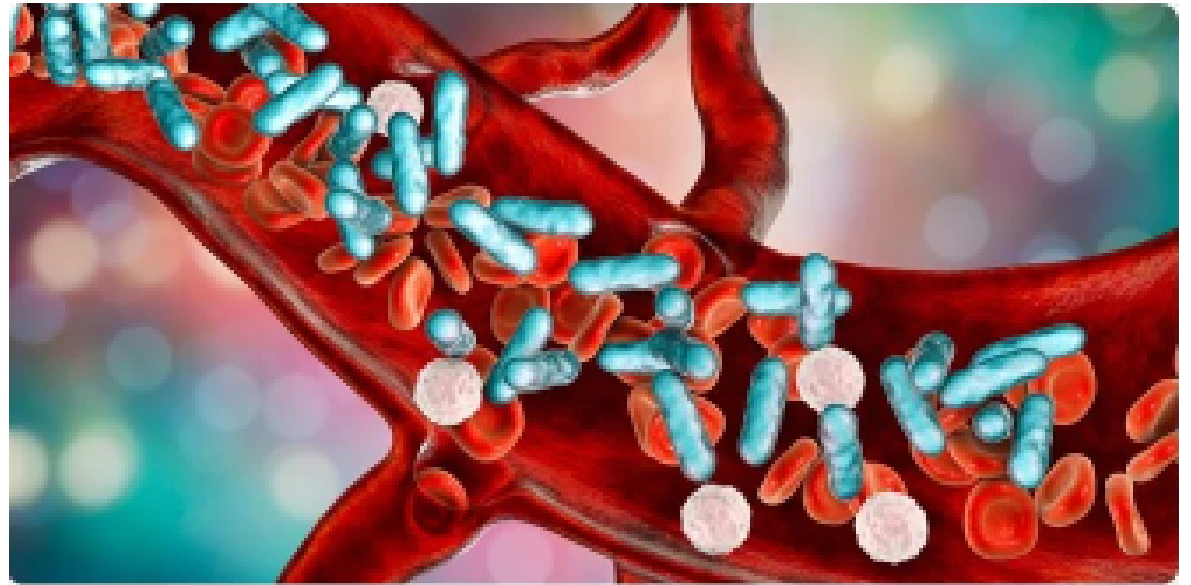
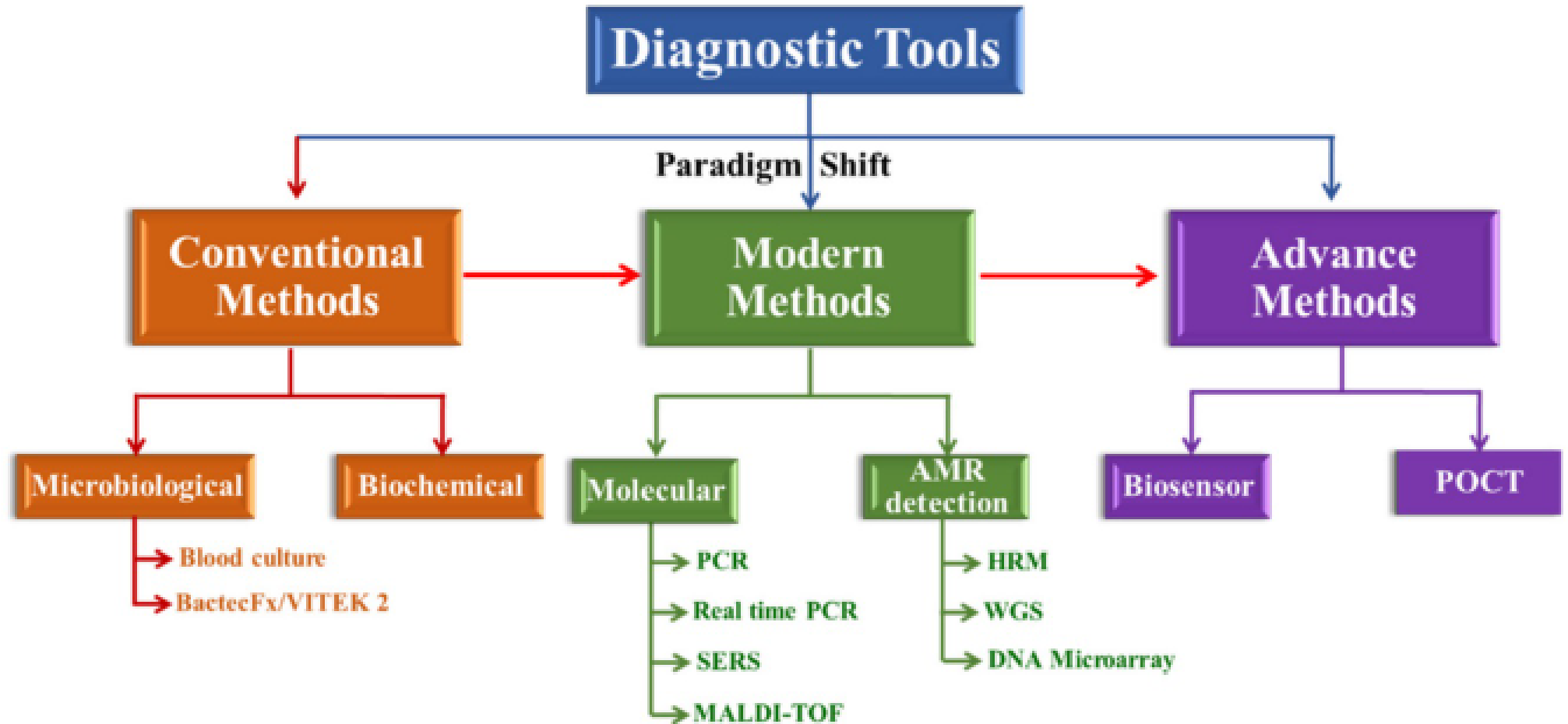


Image Credit: Kateryna Kon / Shutterstock.com



SERS: Surface-Enhanced Raman Spectroscopy

POCT: Point-of-Care Testing

Pre-lab

➤ Sampling

- Time of sampling
 - Fever time
 - Before use of antibiotics
- Blood amount
 - For example 5ml in BACTEC vial
- Sterilization
 - contamination



Laboratory Techniques

- Blood culture
- Routine blood culture
- Automated blood culture

BACTEC



Culture

➤ Preparation medium

- Determination the PH of water
- Use of quality control of medium
- Incubator calibration

➤ BACTEC



Laboratory Techniques

➤ Molecular method

- Conventional PCR
- Real- Time PCR
- MALDI-TOF MS: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

Is a technique for identifying microorganisms by detecting proteins by peptide mass fingerprinting (PMF)

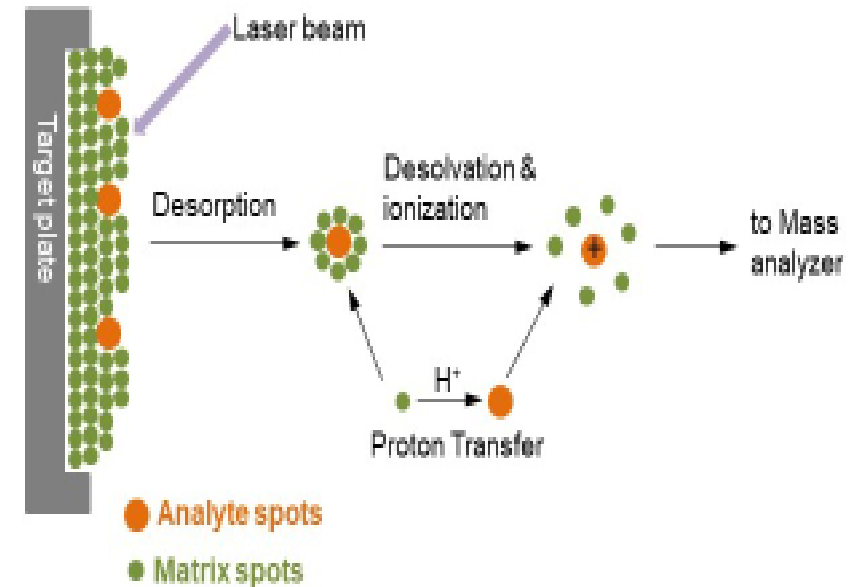
MALDI-TOF MS in routine clinical microbiology laboratories contributes to faster identification of microorganisms compared to conventional methods and can lead to better and faster therapeutic interventions

Laboratory Techniques

MALDI is a protein identification ionization technique in which the analyte crystallizes in a strong lattice matrix crystal that absorbs laser light, allowing it to ionize and desorb from the matrix.

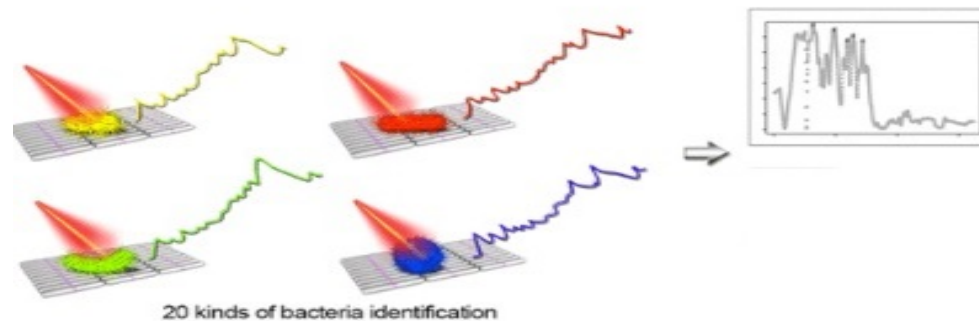
These methods are laborious, expensive, and require chemical reagents that pose a risk to the environment of laboratory workers.

- Another drawback of MS is its restricted capacity in arranging various microbes from the polymicrobial samples.



Laboratory Techniques

- Surface-enhanced Raman spectroscopy (SERS): The Raman dispersing of the objective particles on a superficial layer of metal made of graphene or other different materials.



Laboratory Techniques

➤ Sequencing

- whole-genome sequencing (WGS)
- Next-generation sequencing (NGS), which has revolutionized the biological sciences, is another emerging tool. NGS makes large-scale whole-genome sequencing

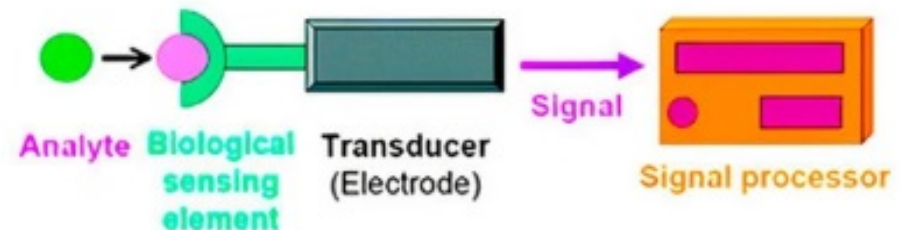


Laboratory Techniques

➤ Advance method

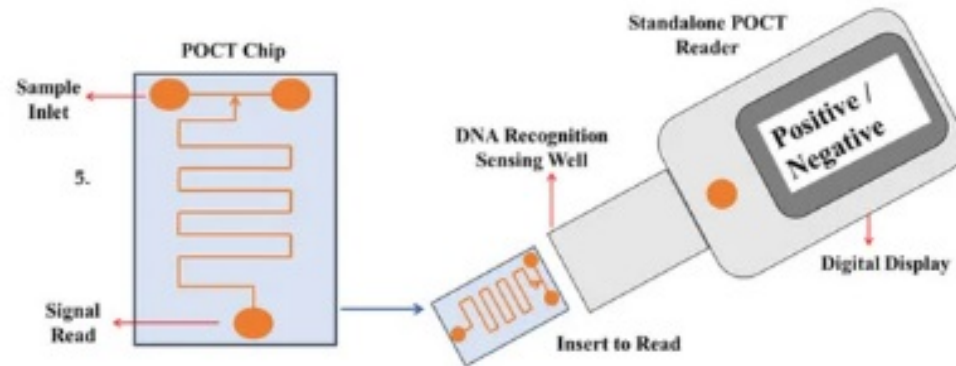
- Biosensors

Because species-specific probes or antibodies give an electrical signal after attaching to their targets, and that signal intensity correlates to the target species total signal, the electrochemical method is the primary criteria on which diagnostic biosensors frequently operate towards identification



Laboratory Techniques

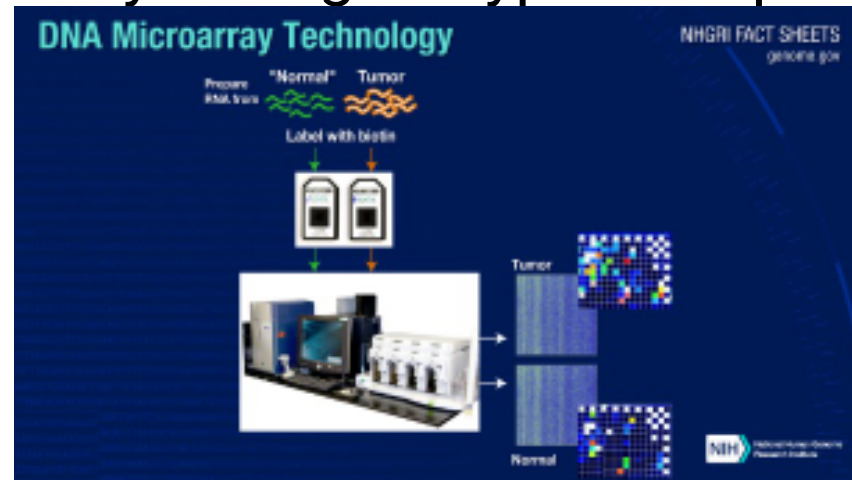
- POCT can also potentially diagnose the assessment of the progression of several proteins' biomarkers (IL-6, IL-10, TNF-a, PCT and CRP) to acute sepsis.



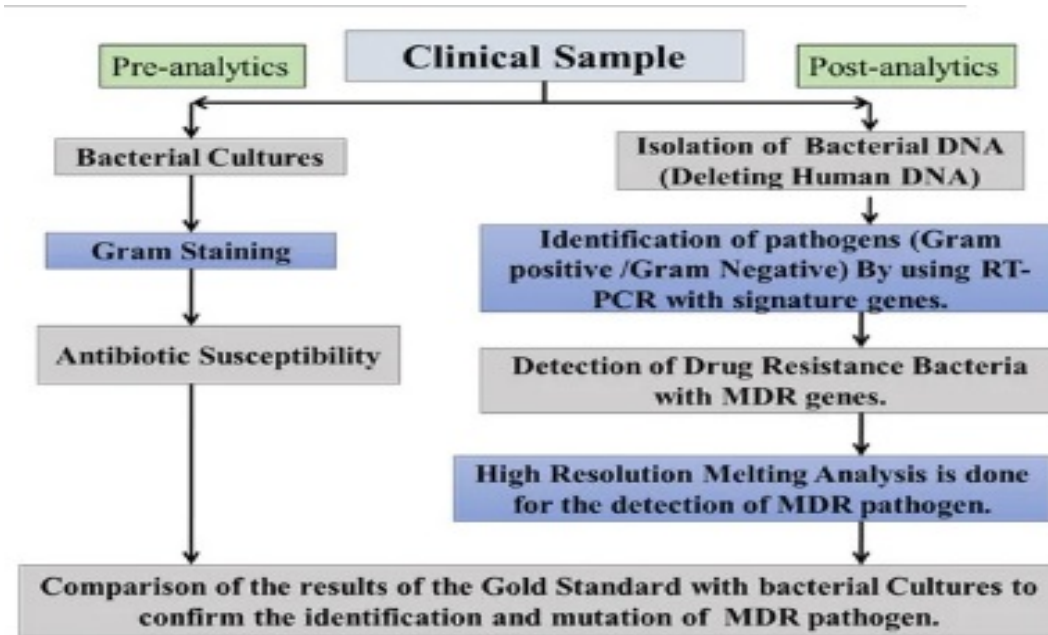
Laboratory Techniques

- DNA Microarray

(also commonly known as DNA chip or biochip) is a collection of microscopic DNA spots attached to a solid surface. Scientists use DNA microarrays to measure the expression levels of large numbers of genes simultaneously or to genotype multiple regions of a genome.



Antimicrobial resistance (AMR)

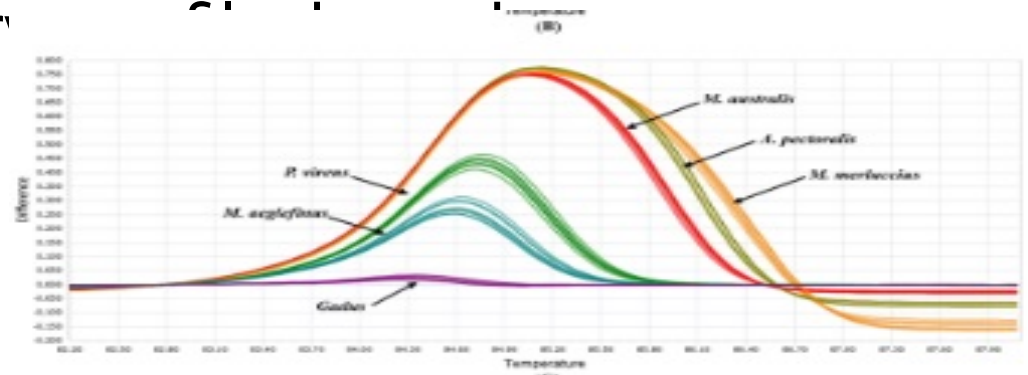


Laboratory Techniques

- High resolution melt (HRM)

High resolution melt (HRM) analysis depends on the detection of differences in melting temperature (T_m) due to the presence of a mutation in a previously amplified target that produces melt curve profiles specific to pathogens.

The size and the sequence of the PCR amplicon is the major reason on which the melting cur



Blood culture or molecular methods

➤ Blood culture

- More time consuming
- Usage of antibiotics
- fastidious bacteria
- Slow growing bacteria
- Anaerobic bacteria



Blood culture or molecular methods

- Rapid
- More sensitive
- Loss of bacteria for antibiogram



Microbial detection methods used in clinical microbiology.

Detection method	Advantages	Disadvantages
Conventional; culture on microbiological media and identification by biochemical tests	<ul style="list-style-type: none"> Sensitive Inexpensive 	<ul style="list-style-type: none"> Lengthy and time consuming process Might require 24–48 h
Molecular based methods (i) Real-time PCR (ii) Multiplex-PCR	<ul style="list-style-type: none"> Culturing of the sample is not required Specific, sensitive, rapid, and accurate Closed-tube system reduces the risk of contamination Can detect many pathogens simultaneously 	<ul style="list-style-type: none"> A highly precise thermal cycler is needed Trained laboratory personnel required for performing the test Loss of bacteria for antibiogram
DNA sequencing	16S rDNA and 18S rDNA sequencing are the gold standards <ul style="list-style-type: none"> Can identify fastidious and uncultivable microorganisms 	<ul style="list-style-type: none"> Trained laboratory personnel and powerful interpretation softwares are required Expensive
Microarrays	<ul style="list-style-type: none"> Large scale screening system for simultaneous diagnosis and detection of many pathogens 	Expensive <ul style="list-style-type: none"> Not suitable for routine clinical use
MALDI-TOF MS	Fast Accurate Less expensive than molecular and immunological-based detection methods	<ul style="list-style-type: none"> Data acquisition and data analysis is time consuming Trained laboratory personnel required High initial cost of the MALDI-TOF equipment Its restricted capacity in arranging various microbes from the polymicrobial samples.

A magnifying glass with a black handle and silver frame is positioned over a cluster of diverse, colorful microorganisms. The organisms include pink spherical bacteria with cilia, yellow rod-shaped bacteria, blue elongated bacteria, and various other shapes in shades of green, orange, and purple. The background is a solid dark blue, and the magnifying glass's lens is centered on the microbial cluster, highlighting them against the darker background.